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Effect of Low-Power He-Ne Laser on Fracture Healing in Rats

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Background and Objective: **Helium-Neon (He-Ne) laser radiation has been found to accelerate fracture healing in laboratory animal models as well as in cultures of cells involved in this process. We investigated the radiological, biomechanical, and histological effects of He-Ne radiation on fracture healing in a rat model.**
Study Design/Materials and Methods: **Sixty-two rats underwent bilateral open osteotomies of the tibiae followed by internal fixation with intramedullary wires. The right leg received He-Ne laser radiation of 0, 2, or 4 Joules every other day for 2 to 6 weeks while the left leg served as a control.**
Results: **Radiological and histological examinations of the osteotomy sites failed to show any enhancing effect of He-Ne laser radiation on the bone healing process. Biomechanically, the irradiated bones of two of the six test groups were significantly weaker than the controls.**
Conclusion: **These results fail to support the previously reported enhancing effect of He-Ne laser radiation on fracture healing.**

Key words: Helium-Neon laser, rats, fracture

INTRODUCTION

Fracture healing is a well-timed sequence of biological events leading to the reformation of bone continuity. The process begins with the formation of a hematoma and continues through the inflammatory stage, callus formation, and finally, the remodelling stage [1]. This process is slow, resulting in prolonged disability and suffering, and it places an economic burden on patients and society.

Several factors have been found to enhance the healing process, including electromagnetic fields [2] and electric stimulation [3], already in clinical use. Low energy He-Ne laser radiation (wave length 632.8 nm) have been reported to have a biostimulating effect on different cells and sub-cell organelles. Rigau et al. [4] found that it enhanced ATP production in fibroblasts, and Passarella et al. [5], using rat liver cells, localized this effect to the mitochondriae. Other investigators [6–9] reported an increase in cyclic AMP and DNA production, although a similar effect was also found with noncoherent red light illumination [6]. Pourreau-Schneider et al. [10] noted mitochondrial hyperplasia and an increase in protein matrix production in fibroblasts exposed to He-Ne laser radiation of 1.2 Joules/cm^2. This effect was even more prominent after multiple exposures.

The effect of low energy He-Ne laser was further investigated using fracture callus as a model. These cells are mesenchymal in origin and are similar under light and electron microscopy to fibroblasts [11]. De Tejada et al. [12] studied the effect of 6 mW irradiation of femural fracture in the rat and found increased vascularity, endothelial hyperplasia, and an early appearance of pre-

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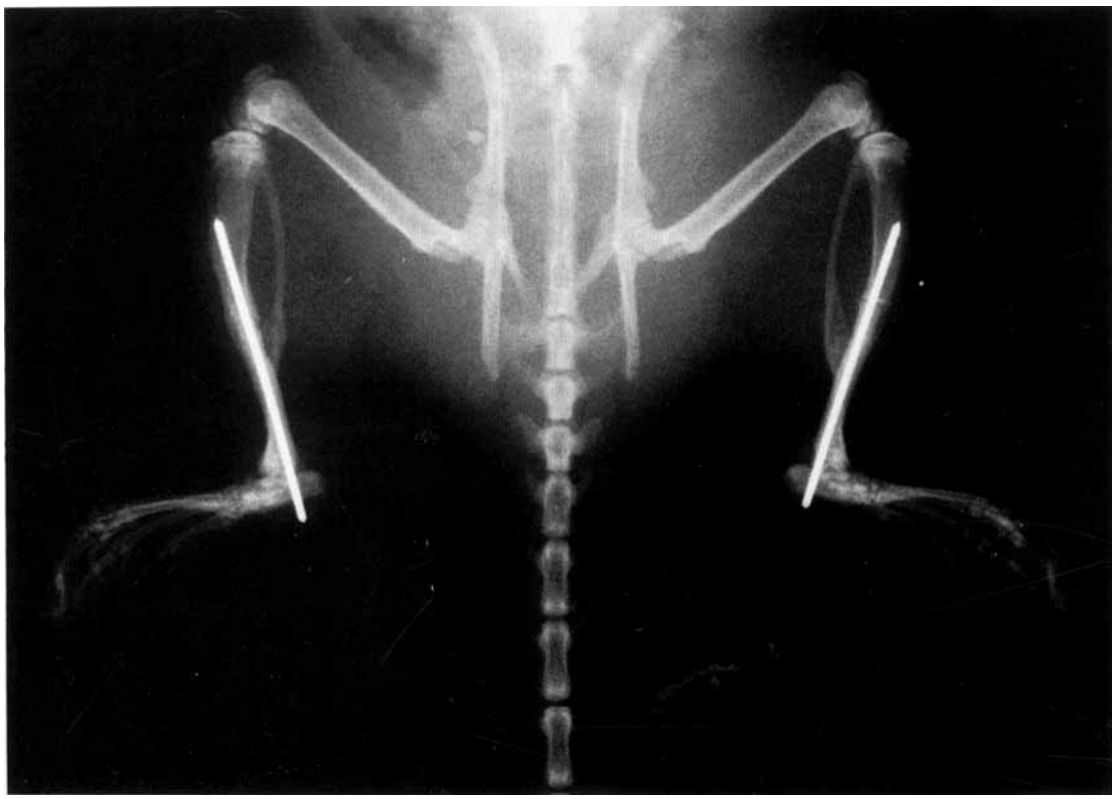


Fig. 1. Radiograph made after fracture induction: the osteotomies were internally fixed using Kirschner wires in an end-to-end opposition.

TABLE 1. Test Groups and Their Subdivision*

Group	Subgroup	N	Time (weeks)	Energy per treat	Total energy
1	a	5	2	0	0
	b	6	2	2	12
	c	6	2	4	24
2	a	6	4	0	0
	b	6	4	2	26
	c	8	4	4	52
3	a	6	6	0	0
	b	9	6	2	40
	c	10	6	4	80

*Energy is given in Joules. N represents the number of rats in each subgroup.

osteogenic cells. Nagasawa et al. [13] observed that He-Ne laser irradiation of 0.19 to 0.76 W/cm^2 for 3 to 5 minutes with a beam diameter of 2 mm yielded superior alveolar bone healing in patients after tooth extraction compared with non-irradiated controls. Trelles and Mayayo [14] found that He-Ne laser irradiation of 2.4 Joules on alternate days for 3 weeks had an excitatory effect on the fracture healing process as determined quantitatively by measurement of the trabecular width. Histologically, the fracture sites of the irradiated callus contained fewer chondroid and more fibroblast components compared to the nonirradiated fractures.

The animal models used by De Tejada et al. [12] and Trelles and Mayayo [14] were created by closed inflection of a long bone in rats and mice, respectively. In none was there radiological confirmation of the fracture architecture and location. Furthermore, the fractures were left unfixed, with possible free movement of their components. In the present work, to gain a better overview of the comprehensive effect of He-Ne laser on fracture healing, a uniform fracture model was created and tested. The energies we used were in the range previously reported to exert an excitatory effect on fracture healing [12,14].

MATERIALS AND METHODS

Sixty-two mature, female Sprague-Dawley rats weighing 225–300 gm served as the test animals. All were anaesthetized by 10% chloralhy-

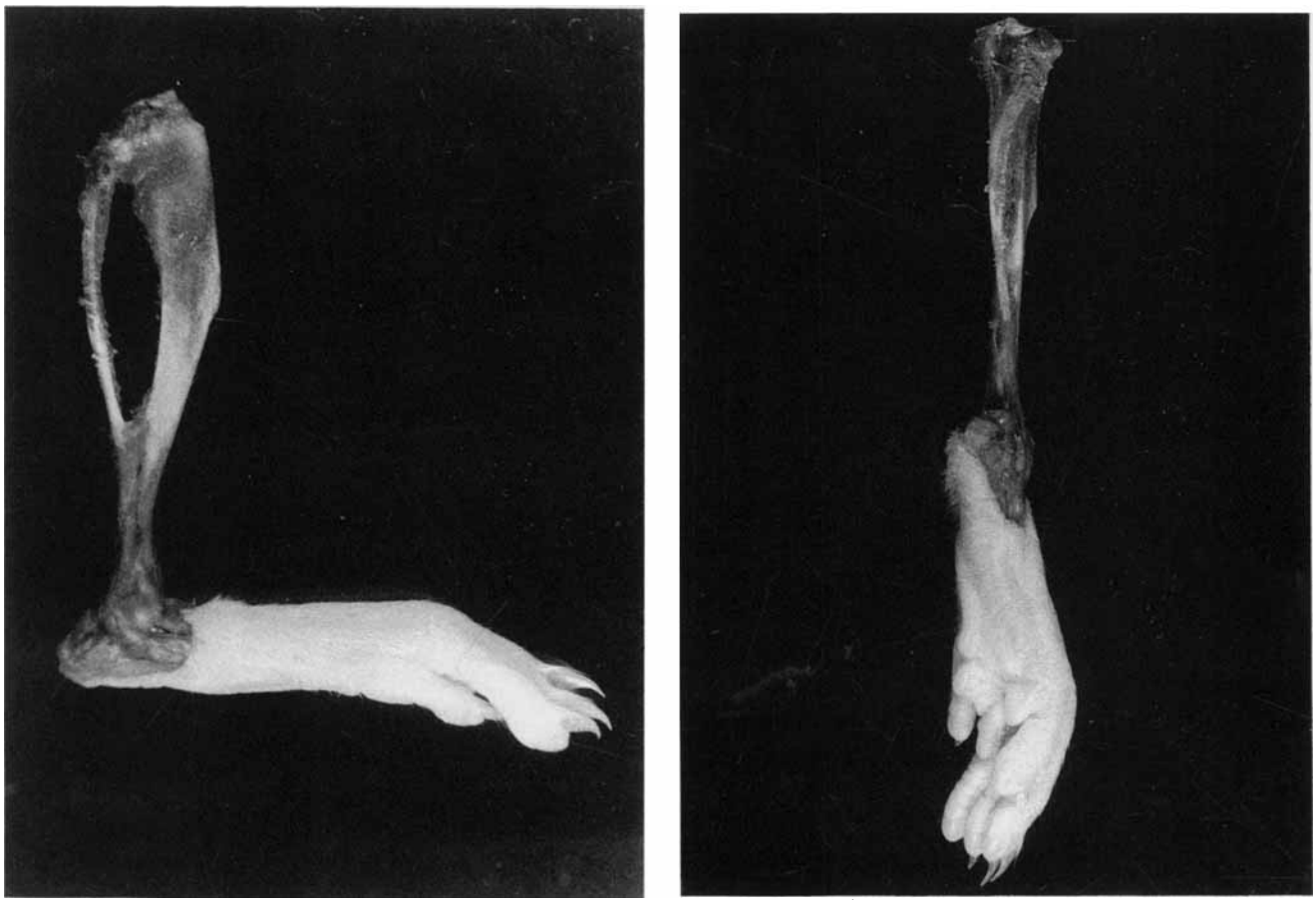


Fig. 2. Gross appearance of the rat tibia. The bending force, represented by an arrow, was applied in (**left**) the F plane and (**right**) T plane.

drate solution injected intraperitoneally at the dose of 40 mg/100 gm BW. Cefazolin (20 mg/100 gm BW) given intraperitoneally was used for antimicrobial prophylaxis. The inner shanks of both legs were shaved and disinfected. An antero-medial approach was used and the tibiae were transversally osteotomized with a ring-cutter type saw. The osteotomies were reduced and fixed by intramedullary 1/32″ Kirschner wires (Fig. 1). The osteotomy sites in the right legs (except controls) were irradiated transcutaneously with He-Ne laser (CW, 10 mW, 632.8 nm) (Uniphase, Stevenage, U.K.) every other day (exposure area 7 mm^2). The rats were divided into three groups and each group was further divided into three subgroups (Table 1). The first group was scheduled to be killed after 2 weeks, the second after 4 weeks, and the third after 6 weeks. The first subgroup of each major group was not irradiated at all. The second subgroup received 2 Joules and the third, 4 Joules at every treatment. The exposures per session of the different subgroups were 0, 28, and 56 Joules/cm^2, respectively.

The rats were killed by an overdose of chloral hydrate. The Kirschner wire was gently removed, and the tibiae underwent the following tests:

Radiographs. All bones were X-rayed and the radiographs were graded according to the following scale:

1. no bony contact, fracture gap more than 1 mm;
2. no bony contact, fracture gap less than 1 mm;
3. partial bony contact;
4. complete bony contact.

Mechanical strength. The bones were tested for bending strength in two planes: the F plane, which passed through the tibia and fibula, and the T plane, perpendicular to it (Fig. 2a, b). The stress-strain measurements were conducted with an Instron machine (model TT-D, Instron Eng. Corp. Canton, MA) using the 4-point bend-

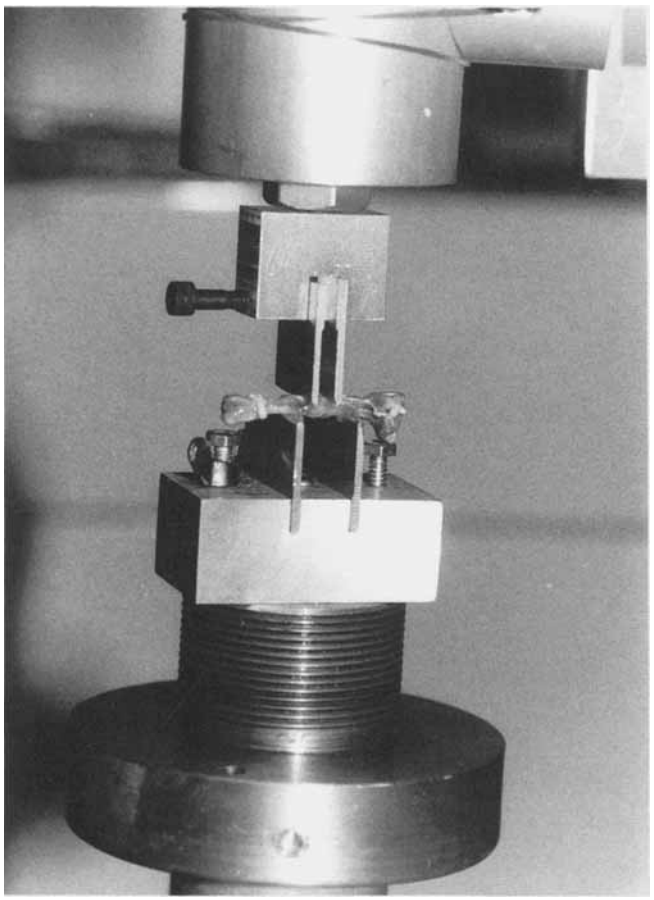


Fig. 3. Four-point bending test. The force was applied and the deflection measured by the Instron apparatus.

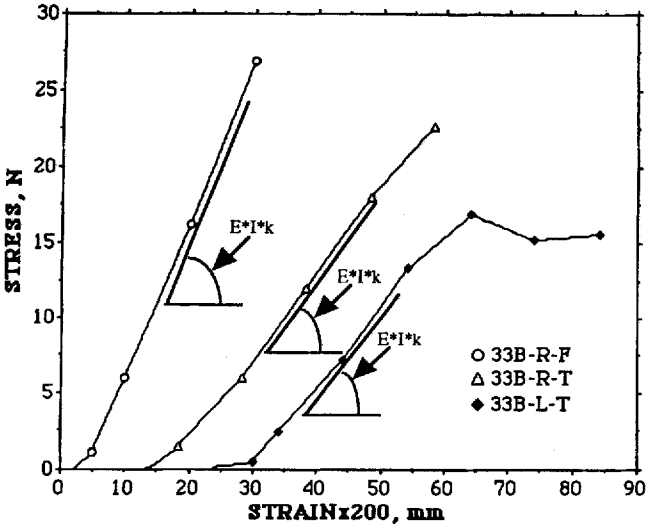


Fig. 4. Schematic stress-strain curves showing the flexural rigidity (E*I*K) of three different bones.

TABLE 2. Radiologic Grading*

Group	Subgroup	N	Time (weeks)	X-ray grading right (mean ± SD)	X-ray grading left (mean ± SD)
1	a	5	2	2.0 ± 0.5	1.8 ± 0.6
	b	6	2	1.9 ± 0.4	2.0 ± 0.6
	c	6	2	1.7 ± 0.5	1.9 ± 0.4
2	a	6	4	2.1 ± 0.6	2.0 ± 0.5
	b	6	4	2.0 ± 0.5	2.0 ± 0.4
	c	8	4	1.9 ± 0.5	2.1 ± 0.5
3	a	6	6	2.6 ± 0.6	2.5 ± 0.7
	b	9	6	2.5 ± 0.7	2.9 ± 0.8
	c	10	6	2.2 ± 0.8	3.1 ± 0.6

*Each osteotomy site was graded on a 1-to-4 scale.

ing method (Fig. 3). For every bone, a stress-strain graph was plotted as seen in Figure 4. The strain was maintained at a rate of 0.5 mm/min, and the full scale of stress applied was 0–40N, remaining in the elastic part of the curve. Results were analyzed by the Wilcoxon rank test.

Histology. Once the biomechanical tests were completed, the bones were immersed in Bouin fixative solution. 1 week later, they were decalcified by Rapid Decal solution (Monville, NJ), sectioned longitudinally, embedded in paraffin, and stained with hematoxylin and eosin. Two different zones of the fracture callus were studied: the external soft tissue next to the osteotomy line and the more distant periosteal reaction zone. In each, the amount of mesenchymal tissue, cartilage, woven bone, and lamellar bone (components of the fracture callus) was determined.

RESULTS

The radiographic grading for the different groups and subgroups is given in Table 2. No significant difference was observed between the irradiated and nonirradiated legs within the same group (i.e., in the rats killed after the same period of time), regardless of the energy dose. There was also no difference between the controlls (no irradiation of either leg, subgroups 1a, 2a, and 3a) and the rats whose right leg was irradiated at different doses for the same period of time. A gradual increase in the quality of healing with time was observed in all the test groups, similarly in the irradiated and nonirradiated legs.

Stress-strain graphs were plotted for all bones and the ratio was measured and calculated. In group 1 (a to c) the fracture areas had no mechanical resistance to the force applied. All other tibiae were tested in both F and T planes. The force/deflexion ratios, representing the stiffness of the osteotomy site are shown in Table 3. The

TABLE 3. Force-Deflextions Values of the Four-Point Bending Test*

		Force—Deflection			
		F plan		T plan	
Group	Subgroup	Rt N/cm	Lt N/cm	Rt N/cm	Lt N/cm
1	a	NU	NU	NU	NU
	b	NU	NU	NU	NU
	c	NU	NU	NU	NU
2	a	1520 ± 730	1240 ± 950	1400 ± 450	1320 ± 840
	b	1630 ± 1020	1340 ± 540	1120 ± 900	1190 ± 570
	c	1110 ± 650	1510 ± 820	670 ± 680	1020 ± 890
3	a	2000 ± 630	1640 ± 800	1920 ± 630	1810 ± 710
	b	1880 ± 1080	2330 ± 1210	1750 ± 1060	2330 ± 1050
	c	2480 ± 1140	2000 ± 680	1680 ± 1280	2280 ± 140

*NU = nonunion (no measurement could be performed). N = Neutons.

only significant results were in group 2c and 3b, where the nonirradiated osteotomy sites appeared to be stiffer than the irradiated counterparts.

The histologic examination failed to show any significant effect of He-Ne laser radiation on the fracture-healing process. No significant change was noted between the irradiated and nonirradiated legs within the same subgroups, or between the nonirradiated (1a, 2a, 3a) and the irradiated rats (2 or 4 Joules). All groups showed a normal pattern of fracture-healing, with a predominance of young mesenchymal tissue in the early stages (Fig. 5), gradually changing into cartilagenous tissue (Fig. 6), and finally bone (woven and lamellar) bridging the gap after 6 weeks (Fig. 7).

DISCUSSION

We investigated the effect of He-Ne laser irradiation on fracture healing in rats for variable periods and at different radiation parameters. The experimental model was an open, controlled osteotomy of the tibia followed by internal fixation with an intramedullary Kirschner wire. We believe that our model is more uniform and reproducible than the earlier closed, manually induced fracture models in mice [14] or rats [12]. The osteotomies were always transverse and in the middiaphyseal area, enabling easy location by simple measurements of the osteotomy site to be irradiated. The intramedullary fixation prevented uncontrolled motion of the fracture components, contributing to the uniformity of the model. The use of rats, whose bones are bigger than those of mice simplifies the surgical procedure and the biomechanical measurements. The biomechanical test served as the major objective index for fracture healing in this study. A torsion test [15] was considered, but the relatively small size and asymmetry of the rat tibia led us to choose the four-point bending test instead. Furthermore, to improve the uniformity of the testing conditions, we distinguished between the F and T planes, assuming that the rigidity in the F (approximately sagittal) plane would differ from that in the perpendicular T plane [16]. No difference however was found in our study, possibly because of the round cross-section of the callus in the osteotomy site.

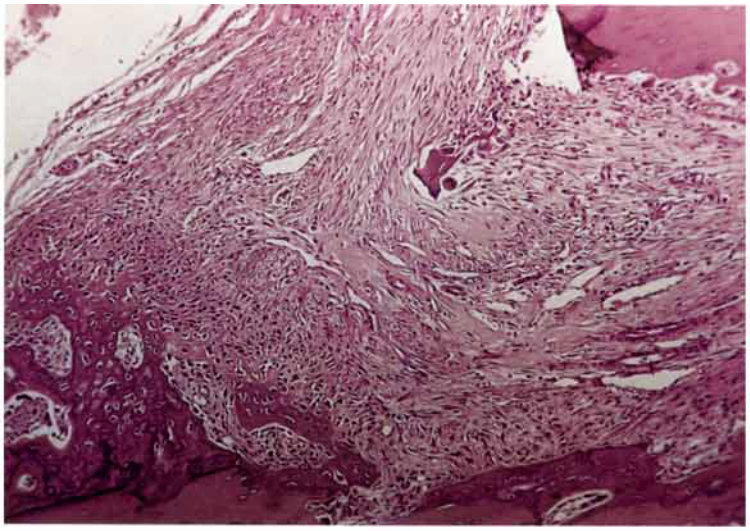
The radiation doses we used were in the range reported to exert an in vivo excitatory effect on fracture healing in mice [14] or rats [12]. Similar radiation parameters were also reported to exert some stimulatory effect on fibroblast cultures [4] and granulation tissue formation in vivo [16]. Other investigators have noted that much lower energies were needed to create a stimulatory effect on cell cultures [10,17] or superficial skin lesions [18]. This may be explained by the need to cross the skin to reach the bone.

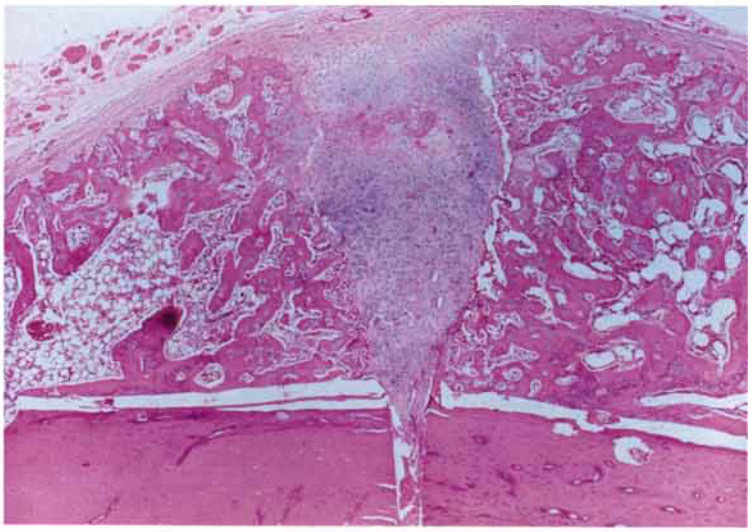
The radiological, histological, and biomechanical results failed to demonstrate any excita-

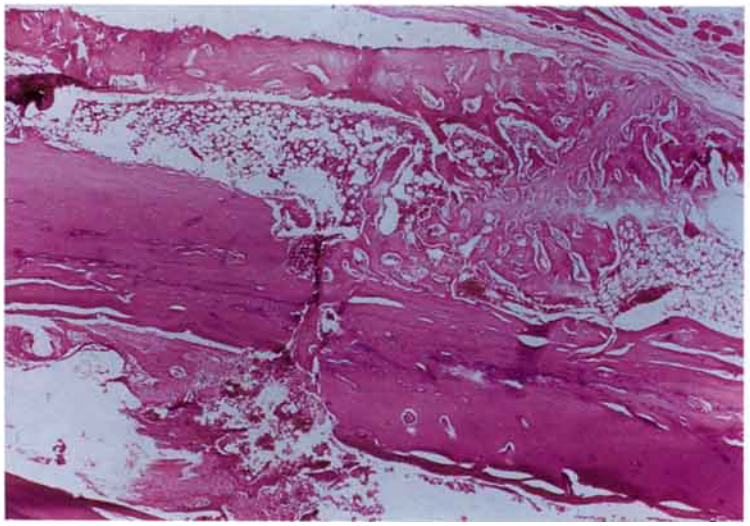
Fig. 5. Microscopic appearance of the callus after 2 weeks. The external soft tissue response region next to the osteotomy is composed mainly of fusiform mesenchymal cells (center of the picture) resembling fibroblasts. In the left side is an area of early bone formation (hematoxylin-eosin, ×100).

Fig. 6. Callus after 4 weeks: the external soft tissue response area is composed of cartilage cells surrounded by woven bone (hematoxylin-eosin, ×40).

Fig. 7. Fracture callus after 6 weeks. Note the bony bridge composed of woven and lamellar bone (hematoxylin-eosin, ×40).







Figs. 5–7.

tory effect of He-Ne laser irradiation on the healing of long bones in rats for the energies tested. The radiological and histological studies are mainly descriptive, but the biomechanical results are quantitative. Although in two out of six test groups the irradiated bones were weaker than the controls', the overall impression is that the tested He-Ne laser radiation dose not enhance fracture healing. These results are in contradiction to those of Trelles and Mayayo [14], who reported faster formation of osseous tissue with a dense trabecular net in the irradiated fractures. Their work, although combining an important electron-microscopy investigation with light microscopy, has two disadvantages. First, their experimental model seems to be inaccurate in the sense that fractures were created by manual inflection, without any radiological confirmation of the fracture architecture, and were left without any internal or external support. Since these fractures cannot be uniform, the conclusions drawn may be inaccurate. Second, these authors used histology as the only parameter for fracture healing. We believe that fracture healing should be regarded more comprehensively, using radiologic and biomechanical parameters as well as histology. This is even more important in cases when histomorphometry is not being used.

More research is needed to investigate the role of different wavelengths and intensities on the fracture healing process, preferebly using more improved experimental models.

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